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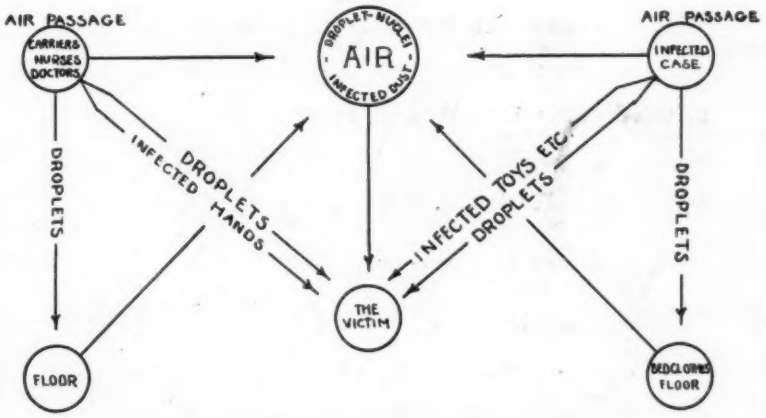

MODES OF SPREAD OF RESPIRATORY INFECTION
(See Review Article by E. G. Klarmann)

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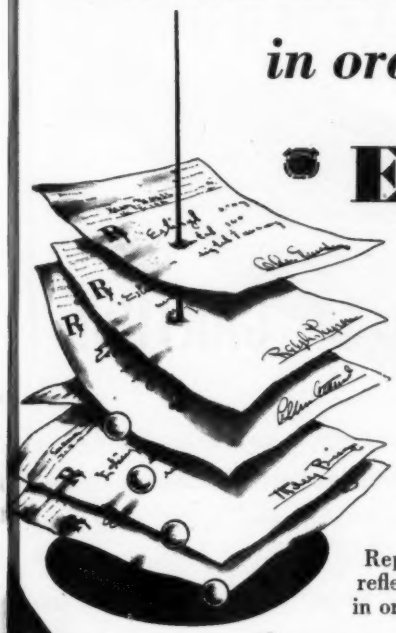

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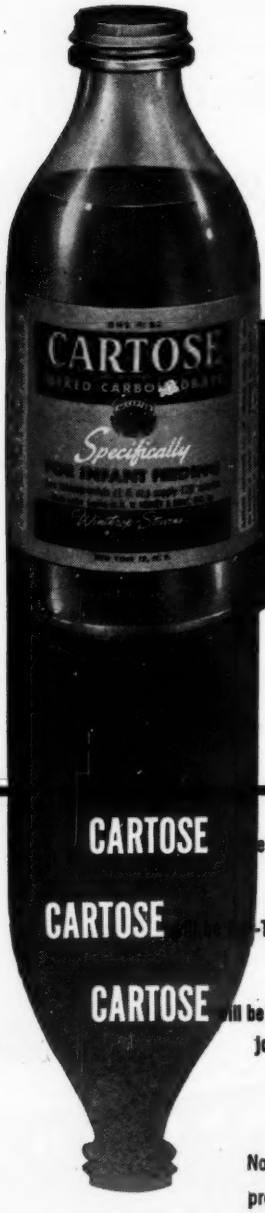

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E D I T O R I A L

ONE OF THE GREATEST NEEDS OF SCIENCE AND HUMANITY

THE editor was quite impressed with an article* appearing in a recent issue of the *Journal of The Franklin Institute.* The authors describe in a most convincing manner the need for a universal language of science, the reason why this need has become so acute, and what steps should be taken to meet this problem adequately.

It is pointed out that Latin, which once was the universal language of men of science, has fallen into disuse for a number of reasons. Revolt against the use of Latin was a part of the humanistic revolt against class privilege and as such it was probably justified. National languages became the language of science, but this is undoubtedly a worse evil. Since 1850 practically no scientific work has been written in Latin.

There are many reasons why Latin should not be replaced as the medium of international exchange, including the fact that it is burdened with a grammatical complexity that makes it cumbersome and difficult.

The need for a universal language of science is keenly felt by anyone who comes in contact with the extensive literature of the world of science. It is next to impossible for one to be capable of translating, let alone speak, three or four national languages and yet one should know dozens, if international barriers to the free exchange of thoughts are to be abolished. How many American scientists today understand Russian? Yet how can the extensive research in Russia be ignored? With German and French the usual languages required, what about important work published in Spanish, Dutch, Norwegian, Danish, Polish, Japanese, Italian and so forth? How can one communicate with a fellow-scientist in these countries with the barrier of language? True indeed is the contention that the lack of an international language of science is a tremendous barrier to world progress. The authors describe several well developed inter-

* Languages for Scincee by Parry Moon and Domina Eberle Spencer, *J. Franklin Inst. 246,* 1-12 (1948).

national languages, including Esperanto, and recommend that one be selected as a secondary language to be used the world over as the language of science. Such steps have been proposed before by world famous scientists but as yet little has been achieved in this direction.

It seems to us that the problem is even larger and more important than envisioned by scientists even though in science it is of major proportions. One of the greatest barriers to world peace today is the failure of different peoples and nations to understand each other, and the greatest obstacle to such understanding is language. This has existed now for centuries with little real effort to approach the problem scientifically. The attempt at a solution by requiring scientists to acquire a reading knowledge of at least two foreign languages is a feeble and ineffectual answer at best for not only are two languages woefully inadequate but the difficulty of mastering the complicated grammar and idiom of two modern languages is tremendous. An added problem for most Americans, at least, is that they do not initiate their study of foreign language until an age when learning a language is difficult. It should be taught in the early years. Psychologists have shown that most children can learn two languages simultaneously with little extra effort and that by learning a second language then they maintain their proficiency in it throughout life.

We can think of no one thing that would hold greater promise of peace and progress than the ability of each person to communicate with any other person regardless of nationality. It is somewhat incongruous to see members of the United Nations Assembly seated with earphones through which a translation of the speech at hand comes. One would think that among the first efforts of the Assembly would be a strong recommendation to each member country embracing the following points:

I. That an international language be adopted throughout the civilized world;

II. That this language be taught simultaneously in all schools together with the native language of the country;

III. That efforts be directed toward the early adoption of this language for all international exchange;

IV. That scientific articles be accompanied by abstracts in the international language with the long range aim of publication in the international language; and finally,

V. That an international committee be established to keep the international language current and adapted to changing conditions so that it would be of maximum usefulness.

Surely in the long run this issue is of far greater importance than some of the trivia now occupying the attention of the world. It might well be that some constructive work on a problem concerning which there should be general agreement might help ease the tension and distrust so rampant in today's deliberations and debate.

Should such a project become a reality the time might come when the international language would become ascendant and eventually be *the* language of the world. Some one language will in time be the only language, this seems a certainty. Shall it be an international language adopted by peaceful means, followed by understanding and eventually a peaceful world union or shall it be one language forced on all others by conquest, destruction, and enslavement?

Scientists by taking strong positive action toward an international language and other opportunities for mutual understanding may still checkmate the terrific evil loosed on the world by the development of the atomic bomb. No longer may we be content with the seclusion of the laboratory nor can we justify social and political indifference among scientists. The hope of the world depends upon how soon international good will can be established.

L. F. Tice

DISINFECTION AND ANTISEPSIS: TRENDS AND IDEAS*

(Part I)

By Emil G. Klarmann, D. Sc.**

Introductory Note

BEFORE proceeding with the subject assigned to me for presentation, may I take the liberty of referring briefly to the purpose of the Gordon Research Conferences which, as I understand it, is not to review published data in any given field of chemistry in a more or less comprehensive manner, but to focus attention upon those recent developments which would seem to offer the greatest promise of stimulating discussion, and thus of suggesting new approaches to existing problems or of opening up new vistas worthy of the chemist's consideration. This introductory statement may serve also to account for any lack of completeness which might be noted in one or another part of this paper. Aside from the fact that it would be utterly impossible to cover every conceivable aspect of my theme within the time period at my disposal, this presentation would not be aided by any wholly uncritical completeness. By the same token, however, a *selective* treatment of any subject cannot help becoming a *subjective* treatment, to a minor or major degree; it is entirely possible or even probable, therefore, that some pertinent developments which to me seem significant may not appear in the same light to others, and conversely, that I may have minimized the signficance of certain developments which would merit emphasis in the opinion of others.

I propose to discuss the trends in the fields of disinfectants, antiseptics and fungicides in the order given. I shall concern myself almost exclusively with chemical materials of synthetic origin, but not with chemotherapeutic agents either of synthetic character (e. g., sulfa-drugs), or of antibiotic character (e. g., penicillin), except where indicated by direct relevance to the subject matter of

* Presented at the Gordon Research Conference on Medicinal Chemistry (American Association for the Advancement of Science), New London, N. H., August 23, 1948.

** Vice-President (in Charge of Research), Lehn & Fink, Inc., Bloomfield, N. J.

this presentation. Admittedly, this is an entirely arbitrary delineation of the project, but one which is dictated both by considerations of expedience and by the framework of my assignment.

Inadequacy of Testing Methods for Disinfectants

Prior to embarking upon a discussion of any particular class of disinfectants, I would like to refer first to the expansion of the rationale of disinfection proper to which the literature of the recent past has contributed very materially.

After the great developments of the latter half of the nineteenth century which furnished the fundamentals of transmission of infectious disease, and established the role of disinfection as an important preventive measure, there followed a rather lengthy period of a seeming formalization of the pertinent ideas; the appearance in this picture of "official" testing procedures, fostered by regulatory agencies and therefore enjoying quasi-legal sanction, further contributed to an oversimplification of the problems involved. Among other things, the classical F. D. A. testing method (Circular No. 198 of the U. S. Department of Agriculture) (1) neatly separates the subjects of disinfection and antisepsis, by setting up *E. typhosa* as test organism to be employed with reference to disinfectant uses on inanimate subjects, whereas *S. aureus* serves in the same function with reference to antiseptic uses, i. e., those on the human or animal body, or in body cavities which can be reached from the outside for antiseptic medication.* The presumption, implied though not expressed, is that *E. typhosa* furnishes an adequate representation of those infective microorganisms whose destruction is aimed at in disinfection, whereas *S. aureus* plays the same role with respect to antisepsis.

The non-existence of any parallelism of germicidal action upon *E. typhosa* and *S. aureus* in the case of a number of coal-tar disinfectants has been recognized by Ruehle and Brewer, the authors of Circular No. 198 (2), while Philbrick (3) demonstrated subsequently a similar situation with regard to *E. typhosa* and a hemolytic *streptococcus*. Klarmann and Shternov (4) showed that in the series of the pure homologous alkyl phenol derivaties, an approxi-

* An exception is supplied in the case of surgical instruments for which *S. aureus* is prescribed as test-organism in ascertaining the disinfectant efficacy of preparations recommended for such use.

mate parallelism of germicidal action upon *E. typhosa, S. aureus, M. tuberculosis* (hom.) and *Monilia albicans* existed up to and including the butyl derivative, but that this relation ceased to exist with the amyl compound.* However, the technical coal tar disinfectants, and notably those of the tar oil or emulsifiable variety, contain varying proportions of alkylphenolic and "neutral oil" constituents (the latter consisting substantially of methyl and dimethylnaphthalenes) which make it entirely hazardous to "guess" the germicidal effect upon any given microorganism from the known effect upon another, e. g., *E. typhosa.*. It should be added here that Circular 198 provides for a so-called "safety factor" in demanding that the dilutions recommended for disinfectant uses should be made to correspond theoretically to 5% phenol rather than be based upon their actual performance under the conditions of the F. D. A. testing method. Aside from the entirely arbitrary character of this requirement, there is, of course, no known reason to believe that increasing the concentration of phenol from 1.25% (or 1:80 which is frequently germicidal for *E. typhosa* under the conditions of the F. D. A. testing technique) to 5%, will yield a disinfectant solution comparable in all respects with one obtained under the same premises from, e. g., a tar oil disinfectant containing a substantial proportion of neutral oil and a minor one of phenolic constituents. The F. D. A. testing method is an outgrowth and refinement of the Rideal-Walker procedure (6); although both these procedures, and particularly their mathematical results, viz., the "phenol coefficients," are intended to apply only to substances chemically related to phenol, the literature, even that of the most recent past, is replete with references to the evaluation of disinfectants and antiseptics by the F. D. A. method, bearing no chemical or other relation to phenol whatever (7).

This matter of an assumed resemblance to phenol of various antibacterial substances merits further brief consideration because of the ill-founded references to it frequently encountered in the literature.

From the point of view of their germicidal performance, two disinfectant substances may be said to be similar in action if they behave similarly upon dilution; and the latter condition is satisfied

* The "quasi-specific" regularity in the case of halogen alkyl phenol derivatives is referred to in the papers by Klarmann, Shternov, Gates and Cox (5).

only when their concentration exponents are the same. On the assumption (for which both theoretical and experimental support has been obtained in a number of instances) that the death of bacteria under the action of a disinfectant, proceeds in a manner comparable to that of a chemical reaction of the first order (8, 9, 10, 11, 12), the equation $c^n t = a$ (*const.*) will apply in which c is the concentration, n the concentration exponent and t the time of disinfection. For instance, let the concentration exponent equal 1.0; in this case, when any concentration of the disinfectant is doubled, the lethal time, for a standard size inoculum will decrease 2 times, i. e., by one-half. According to Jordan and Jacobs (13) the concentration exponent n for phenol is 6; therefore, doubling its concentration should result in a 2^6, i. e., 64 fold increase in the velocity of disinfection, (or, conversely, reduce the time of disinfection to the corresponding fraction of the original). The compilation of concentration exponents by Rahn (14) indicates a wide range among the various disinfectant substances listed by him.

Under the conditions of the F. D. A. testing method, there exists (or is presumed to exist) *one* definite concentration of phenol and *one* of the disinfectant substance under test producing the death of the inoculum at the same time, viz., ten minutes; the phenol coefficient is calculated from these concentrations. However, it neither follows, nor is it true that these two substances bear the same relation when other times are chosen for the end-point. A constant relation can be expected over a range of dilutions only when the concentration exponent n is the same for the disinfectant standard and the disinfectant under test; if the exponents are not the same, the relationship expressed by the phenol coefficient will hold only for the respective dilutions effective in the ten minute period, but it will not furnish any information concerning this relationship with respect to other dilutions.

That these facts must be kept in mind even in the case of presumably closely related compounds follows from a recent study by Withell on p-chloro-m-cresol (15). From the chemical viewpoint, this substance must be regarded as a phenol derivative; therefore, the propriety of using phenol as a test-standard would seem to be beyond question. Nonetheless, Withell found the concentration exponents for phenol and p-chloro-m-cresol to be respectively 4.55 and 8.3, a fact which would suggest that there is indeed some ques-

tion concerning the unqualified use of phenol as a standard of comparison for p-chloro-m-cresol.

It is readily understood, therefore, that any implied assumption of an adequate similarity between the phenol standard and a given disinfectant is very likely to be in disagreement with facts, and that, therefore, the phenol coefficient may tell only a fragmentary, if not an altogether incorrect story (16).

Of course, nothing that has been said thus far reflects upon the fitness of phenol for use in standardizing the bacterial test cultures; phenol has had a long record of satisfactory performance as an aid in selecting strains of proper resistance for testing disinfectants and antiseptics.*

New Findings on Transmission of Respiratory Disease

This statement of random thoughts on the subject of testing disinfectants has been made because its consideration is deemed proper in connection with an extensive series of recent investigations originating in England and continued here, and bearing upon the problem of transmission of infections via the naso-pharyngeal route. The second world war provided an excellent opportunity of studying and combating the incidence of cross-infections in hospitals and in barracks; it is axiomatic that anything learned about such matters in hospital wards should apply directly to the understanding of the spread of respiratory infections in general since the prevention of such infections in schools, homes, etc., depends upon the same basic principles.

For a number of years prior to 1934 it was generally accepted that acute respiratory diseases were spread largely by contact; the latter term is understood, in this connection, to mean either direct transmission as e. g., by kissing, or indirectly, e. g., by contaminated hands, infected surgical instruments or other material objects. Since then, however, it became evident that the air of enclosed spaces could

* The recent observation by Flett, Haring, Guiteras and Shapiro (167) to the effect that it is possible to produce an increase in the minimum germicidal concentration of phenol (acting in 10 minutes) from 1:80 to 1:65 with respect to *E. typhosa*, and from 1:65 to 1:55 (or 1:50) with respect to *S. aureus* (by using charcoal or ferric chloride in the transfer tubes) does not affect the position of phenol as a satisfactory standardizing agent. In the first place, the "inactivation" is of the order of only around 20%, and in the second place this phenomenon may be relevant to some other aspect of the disinfectant problem, but hardly to that of standardization of test cultures.

become badly contaminated with a variety of pathogenic micro-örganisms, and this, in turn, opened up the question of the comparative significance of the contact and aerial routes.

If the transmission of disease takes place via the aerial route the following three possibilities must be considered:

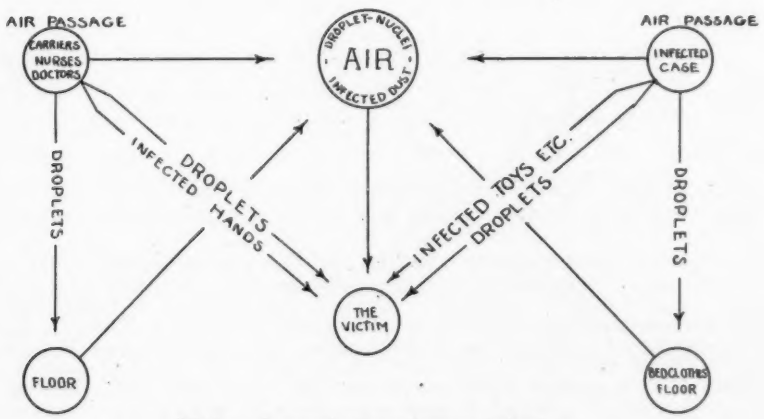
1) Droplet infection, implying direct impingement of the ejected infective matter upon, e. g., the mouth, the conjunctiva, the open wound, etc.;

2) Infection by droplet nuclei, implying, e. g., inhalation of the minute residues of evaporation from droplets which because of their smallness and lightness may remain suspended in the air for long periods of time;

3) Infection by contaminated dust representing a secondary reservoir of infective particles which originate on the floors, also on bed clothes, etc., and which may remain suspended in the air for comparatively short periods of time, particularly following some mechanical activity, such as sweeping.

The following diagram (Fig. I) taken from a comparatively recent memorandum of the British Medical Research Council (17) illustrates this set of factors contributory to the spread of respiratory infection.

FIG. I



Modes of spread of respiratory infection.

Now as to the problem of the relative significance of the three factors mentioned, the following may be of interest:

Hare and Mackenzie (18) determined the rate of expulsion of liquid particles from the mouth and nose by speaking, coughing, sneezing and blowing. Using a special holding arrangement, they placed five agar plates at the angles of 0°, 22.5°, 45°, 67.5° and 90° relative to the subject's mouth. They found that the great majority of microorganisms are contained in droplets of fairly large size whose trajectory depends upon the resultant of the expelling force and of the force of gravity acting perpendicularly to their initial motion. The trajectory is flatter in the case of violent methods of expulsion than in that of talking. As shown in the following Table I, in the case of blowing, the proportionate majority of the organisms are to be found on the 67.5° plates, in that of coughing on the 67.5° and 45° plates, in that of sneezing on the 45° and 22° plates, and in that of talking on the 22.5° and 0° plates, i. e., more directly under the mouth. In absolute terms, the greatest number of organisms is expelled by sneezing, the smallest by talking. Few organisms reach the atmosphere when the subject is breathing quietly.

The ultimate destination of the expelled particles depends also upon the position and the surroundings of the donor. Thus when lying in bed, a high proportion of particles will impinge mostly upon some surface below the mouth. When standing upright some of the

TABLE I

AVERAGE NUMBER OF COLONIES PER PERSON ON PLATES EXPOSED AT DIFFERENT ANGLES IN RELATION TO THE MOUTH (HARE AND MACKENZIE)

Position of Culture Plate	Talking		Coughing		Blowing		Sneezing	
	Average	% of Total	Average	% of Total	Average	% of Total	Average	% of Total
0°	28.1	30.0	6.7	4.9	1.3	2.6	355.8	7.5
22.5°	39.1	41.8	8.9	6.5	1.6	3.2	1,636.0	34.4
45°	16.2	17.3	60.1	43.9	8.0	16.0	2,425.6	51.1
67.5°	8.4	9.0	52.9	38.7	27.0	54.2	262.8	5.5
90°	1.8	1.9	8.2	6.0	12.0	24.0	71.3	1.5
Total ..	93.6	100.0	136.8	100.0	49.9	100.0	4,751.5	100.0
Number of subjects	39		20		3		9	

droplets will fall to the ground, some upon the clothes, some will lose moisture by evaporation and turn into free floating droplet nuclei. In this connection it is noteworthy that according to Davis (19), droplets above 7 microns in diameter are removed in the process of inhalation by the nose while one-half of those of 3 microns reach the lungs which are also penetrated by all those 1.5 microns or smaller.

Dust as Carrier of Streptococcal Infection

As to some earlier work on the role of dust in the transmission of respiratory diseases, it may be mentioned that as early as 1917 Stillman called attention to the presence of pneumococci in dust, and that in 1919 Eagleton found meningococci on the premises housing carriers (20). The participation of dust in the transmission of hemolytic streptococci was considered by several investigators.

In 1935 Cruikshank (21) reported that the types of hemolytic streptococci infecting burns were the same as those present in the air and in the throats of certain of the ward patients. Soon thereafter, White (22), and later Cruikshank and Godber recognized the role of infected droplets and dust in puerperal fever (23) when suggesting that scarlet fever, tonsillitis and pharyngitis could be acquired via the air route from puerperal fever cases; the same aspects in streptococcal complications of measles and influenza were studied by Cruikshank and Muir (24). Cruikshank and his collaborators realized that while the primary source of respiratory pathogens is, of course, the infected patient or carrier, the secondary reservoirs in which such disease agents accumulate are furnished by floor dust, bed clothes, etc. These findings were confirmed and extended by other English and Canadian investigators, including Van den Ende, Thomas, Hare and their collaborators (25, 26, 27, 28, 29). The following finding reported by Thomas is illustrative (26). Thomas examined the floor dust collected in one morning in one hospital ward during an outbreak of streptococcal sore throat and follicular tonsillitis; there were twenty-two patients in a twenty-six bed ward, nineteen of whom exhibited streptococci in their throats. The total weight of dust was one ounce, reflecting an acceptable degree of cleanliness; yet it contained the enormous number of over one-hundred million of streptococci which were distributed as shown in the following Table II.

TABLE II

DISTRIBUTION OF HEMOLYTIC STREPTOCOCCI IN HOSPITAL DUST (THOMAS).

Ward (16-bed section)	16,261,000	Office	1,041,000
Ward (10-bed section)	23,323,000	Linen room	939,000
Cubicle 1 (F)	17,020,000	Staff day-room	329,000
Cubicle 2 (non-clinical case)	1,173,000	Day-room (transit only)	320,000
Surgery (E. N. T. dept.)	160,000	Entrance corridor (kitchen)	1,171,000
Main corridor	1,411,000	Annex (toilets, baths, etc.)	38,745,000

In this connection mention may be made of the work, in this country, of Buchbinder and collaborators (30, 31) who carried out studies on changes occurring in the Group A of hemolytic streptococci sprayed into simulated room environments. These organisms were found to survive long periods of time without loss of pathogenicity, as verified by their virulence for mice.

A considerable number of publications by American investigators helped to clarify the problem of dispersal of respiratory pathogens in general, and of hemolytic streptococci in particular. Hamburger and coworkers made a number of significant observations (32, 33, 34, 35) over a considerable period of time among which are the following:

In the acute stages of streptococcal sore throat or scarlet fever, over eight per cent. of saliva specimens contained hemolytic streptococci of the same serological type as found in the throat; when present in saliva their number per cubic centimeter varied from about one hundred to several million.

Sulfonamide drugs administered in doses of four to six grams over a period of one to fifteen days did not decrease the number of hemolytic streptococci in saliva.

As to the intensity of their dispersal, more organisms are expelled from the upper respiratory tract by blowing the nose than by sneezing or coughing; in fact millions of beta hemolytic streptococci are expelled by a carrier exhibiting a positive nose culture, with

a single nose blow. This produces a threefold effect; it contaminates the handkerchief, it contaminates the hands, and it allows the escape of a number of streptococci into the air. As the mucus on the handkerchief dries, more organisms are distributed into the air, while the contaminated hands in turn contaminate personal clothing, bed clothes and other objects touched by them; all this creates secondary reservoirs which become the major source of air-borne hemolytic streptococci.

The observation made by Gordon several years prior to Hamburger's investigations, viz., that scarlet fever patients discharged with rhinitis are more likely to transmit the disease to others than those without rhinitis, fits into this picture (36).

In Hamburger's opinion, the "direct hits" by streptococci ejected by sneezing or coughing are probably of lesser importance in setting up infections, as is also the inhalation of droplet nuclei, even though a few hundred organisms are considered sufficient to cause streptococcal sore throat.

Although Coburn (37) suggested that in certain carriers some strains of streptococci undergo biological changes which make them communicable (in contrast to others which remain non-communicable), Hamburger believes that the difference between carriers resides in the number of microorganisms which they can expel. The original papers should be consulted for the substantial clinical support of this opinion. Incidentally, he points to the essential role played by hemolytic streptococci in rheumatic fever which renders the prevention of streptococcal infection a matter of life and death, and not merely one of discomfort or lost man-days.

Other extensive investigations on the transmission of respiratory disease in army barracks by Loosli, Lemon, Wise and Robertson (38), employing special techniques of analysis of the bacterial content of air, bed clothes, wearing apparel and floor dust, support the contention that excretions expelled from the upper air passages contribute to the heterogeneous bacterial population in the environmental air and dust which is involved in causing infection of the respiratory tract following inhalation (39). Since beta hemolytic streptococci are readily isolated and typed, they were used as representative indicators of disease agents of the respiratory tract. Dust cultures collected from over 1500 beds were 23 percent positive for streptococci in winter, and 75 percent positive in spring; 94 per-

cent belonged to group A, 5.8 percent to group C, while groups B or G were encountered only during the winter months.

The amazing capacity of survival of streptococci in blankets is shown by the fact that after six weeks of storage, fifty percent of the blankets were still positive for streptococci. When ten pairs of the most heavily contaminated blankets were stored at 70 to 85°F for over three months, all yielded positive streptococcus cultures at the end of this period.

Streptococci recovered from the dust of floors or of bed clothes did not show any loss of their ability to lyse fibrin when compared with strains freshly isolated from noses or throats (40). Repeated demonstration in the environment, viz., floor dust, bed clothes, etc. of large numbers of the same types of streptococci as found in the noses and throats of the carriers indicates strongly that inhalation of the dust borne pathogens furnishes an important mode of infection and its spread. Low humidity favors a longer survival of streptococci (41).

Incidentally, hand shaking by nasal carriers may transfer thousands of organisms with a single hand shake (the largest number observed being 48,000). A hospital epidemic affecting 150 patients was traced to a food handler from whose hands several millions of hemolytic streptococci were recovered by culturing.

Dust-Borne Transmission of Other Respiratory Infections

The role of dust in the transmission of diphtheria is the subject of an inquiry by Wright and coworkers (42, 43, 44). Virulent diphtheria organisms were recovered in large numbers in the floor dust of diphtheria wards; they were found to be capable of surviving in dust, *in vitro,* for three months while retaining their virulence, as shown by the following Table III.

The significance of the presence of pneumococci in dust was stressed by Stillman (45) who recovered Types I and II from the floor dust of homes in which the corresponding cases of pneumonia occurred. Pneumococci were found in viable form in dust one week to one month following their dispersal (46). Recently Hodges and McLeod (47) reported that the pneumococcal types found in the dust of barracks and school rooms reflected the prevailing types observed in the throats of carriers using these premises.

TABLE III

SURVIVAL OF DIPHTHERIA ORGANISMS IN DUST (WRIGHT ET AL.)

First examination				Second examination				Length of survival
Date			Result	Date			Result	
Oct.	7	G	moderate no., M few	Oct.	14	G	numerous	7 days
Oct.	7	I	few	Oct.	14	G	and I few	7 days
Oct.	7	G	few	Oct.	14	G	few	7 days
Oct.	21	I	very numerous	Nov.	22	I	(virulent)	1 mth
							moderate no.	1 mth
Oct.	21	G	very numerous	Nov.	20	G	numerous, I few, both	1 mth
							virulent	1 mth
Oct.	21	G	very numerous	Dec.	18	G	moderate no., virulent	2 mths
Oct.	21	G	numerous, I and M few	Dec.	21		Negative	
Oct.	28	G	numerous, I few	Dec.	10	G	Moderate no., virulent	6 wks.
Nov.	12	G	numerous	Mar.	3	G	few, virulent	102 days
Nov.	19	G	moderate no.	Mar.	3		Negative	

G=gravis,
I=intermedius,
M=mitis.

While the investigations of Hart and collaborators (48, 49) concern themselves primarily with the contamination of air in operating rooms they are generally significant insofar as they refer to the occurrence in the air of *S. aureus* (hemolytic) of nasopharyngeal origin. The sedimentation of this virulent pathogen upon open wounds or upon sterile supplies, as verified and followed by the observation of settling and culturing on open plates, has been held responsible for over 90% of infections in supposedly clean operative wounds. While bactericidal ultra-violet irradiation of the contaminated air has been found effective as a remedial measure. no reference

is made to any attempts of controlling recontamination of the air by the sedimented infected particles. By analogy with the conditions obtaining in the spread of streptococcal infection, it would seem fair to assume that the sedimented portion of staphylococci deserves the same attention as the suspended portion.

In connection with operating room procedure, brief reference may be made to the observations of Bourdillon and Colebrook (50) who accuse infected surgical dressings of releasing bacteria-laden particles into the air and thus of contributing to the spread of infection.

The tubercle bacillus may be regarded as being the hardiest of the common respiratory pathogens. Its capacity of survival outside the human body under conditions which may be destructive to other pathogenic microorganisms points to the secondary reservoirs as being of particular importance in the spread of pulmonary tuberculosis. Indeed it has been known for some time, e. g., from the studies of Augustine (51) and from those of Neufelt (52) that individuals affected by open pulmonary tuberculosis produce an extensive contamination in their surroundings, among other things, in floor dust and bed clothes, that tubercle bacilli can survive under such conditions for considerable periods of time, and that they can be redispersed from them into the air. According to measurements carried out by Duguid (53), the bulk of droplets expelled by coughs—(some of which may produce as many as 40,000 tubercle bacilli) are large enough to fall rapidly to the nearest surface. Therefore, it is fair to assume that the chief source of air-borne tubercle bacilli is the secondary reservoir of floor dust and bed clothes, although transmission by contaminated hands cannot be disregarded as a possibility of lesser significance.

Influenza virus dried on fabrics or glass has been recovered a week or more following experimental contamination of such objects, according to Edwards (54). A high potency strain of influenza virus (Melbourne) such as may be assumed to occur in pandemic disease, when mixed with mucus and dried in air, may retain its lethal potency for embryonated eggs for as long as forty-five days. From this it is concluded that the transmission of epidemic and pandemic influenza through the agency of dust and dry fomites is a distinct possibility, not to be ignored in the practical control of these diseases (55).

Review of Air Disinfection

What is the status of control of air-borne infections? As is known, a tremendous amount of work has been devoted to the study of the disinfection of air by such means as germicidal ultra-violet radiation, disinfectant sprays and aerosols, and disinfectant vapors. Emphasis upon these modes of combating respiratory infection reflects the belief in the importance of the air as the primary vector of pathogenic microorganisms (56, 57, 58). It would be impossible to discuss with any degree of thoroughness, even a representative portion of the many investigations relating to the three modes of air disinfection mentioned. However, the following brief review may be sufficient for the purpose of this presentation (59).

As to germicidal ultra-violet radiation, its effectiveness decreases with increasing humidity above the range of 55 to 60 percent. It is more active against the smallest particles, such as droplet nuclei, than the larger ones, such as dust or lint which may provide a coating impermeable to the bactericidal wave lengths. This kind of radiation is damaging to the skin and the conjunctivae, therefore requiring care in the installation and operation of the lamps. It was found to perform particularly well in operating rooms. While favorable results were observed in controlled school installations, the extent to which the procedure should be carried out is still uncertain.

The combined ultra-violet irradiation of floors and upper air spaces in barracks caused a 19 percent overall reduction of respiratory disease and a 24 percent reduction of streptococcal diseases (60). While the total bacterial content of the air and of the dust from floors and blankets was reduced by irradiation and dust suppression (oiling), the streptococcus content of floor dust was not changed. It is inferred from these findings that the general use of ultra-violet radiation in barracks, for the control of air-borne infection, is not warranted at this time.

Of the other methods of air disinfection mentioned, considerably more attention is being devoted now to disinfectant vapors than to germicidal aerosols (61, 62, 63, 64, 65), although it is claimed that the latter are capable of producing a combined effect, i. e., both as disinfectant space sprays and as surface disinfectants (66). As to the germicidal vapors, of a considerable number of materials (including several glycols) studied (67, 68, 69, 70, 71, 72), triethylene

glycol vapor appears to be most satisfactory because of its comparatively greater germicidal and virucidal effectiveness, its low toxicity and corrosiveness, and its freedom from odors. It is most active at relative humidities of 30 to 35%. Its effect upon small particles (droplet nuclei) is better than upon dust particles. Its advantage over ultra-violet radiation resides in its permeation of all parts of a room, rather than that of the upper air alone. Its general utilization awaits the development of practical means of effecting and controlling vaporization of triethylene glycol (73, 74).

Volatile chemicals other than the glycols have also received consideration. Among them are triethanolamine, mandelic acid (75), also the homologous series of aliphatic alpha-hydroxycarboxylic acids (including glycolic, lactic, alpha-hydroxyvaleric, etc.) and the monoesters of maleic acid, all of which were found to be effective in destroying the organisms of sprayed saliva (*S. salivarius* and others) and are, therefore, of potential interest in aerial disinfection (76).

However, as indicated above, the measures aiming at air disinfection are derived substantially from the premise of a *direct* dissemination of infective droplets (or droplet nuclei), i. e., from donor to recipient. On the other hand the idea appears to be gaining ground that the *indirect* spread of bacterial infections of the respiratory tract, by inhalation of dust borne bacteria, is considerably more important than the direct inhalation of infective particles (28, 44) (although in the case of virus infections the relative importance of the direct and indirect routes cannot be determined at this time). It is fitting, therefore, to inquire into the methods of controlling the dust-borne route of infection, and the successes attendant upon their employment.

Control of Dust-Borne Infection

It was logical to assume that measures aiming at the suppression of dust should be of help in reducing the number of infective particles due to sweeping or other forms of activity. In keeping with this, the application of light paraffin oil (spindle oil) was resorted to extensively. Oiling is applicable by saturation to wood, but not to concrete, linoleum or waxed surfaces. Special methods have been developed also for the treatment with oil of blankets, bedding, etc., by means of an oil emulsion made with a neutral emulsi-

fying agent; such applications are said to be effective for some time and to resist a number of launderings.

Details as to the effect of the oiling procedure may be found in a recent paper by Loosli (77). While oiling of fabrics and floors tends to reduce the dispersal of dust, the studies on the control of respiratory disease by this method have not given any wholly conclusive results. This is illustrated by a selection of the following findings:

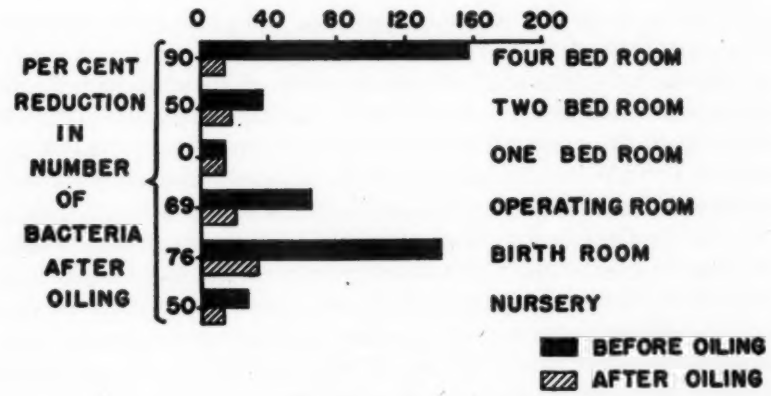
Wright, Cruikshank and Gunn (78) who studied the incidence of streptococcus infections in children's measles wards found that the incidence of cross infections in an oiled ward was about one-third that of a non-oiled control ward. In an investigation of army barracks, Loosli and Robertson (79) found that the incidence of acute respiratory infections (one-half of which were due to hemolytic streptococci) in oiled areas was about 50 percent of that in the control barracks. A joint study by the Commissions on Acute Respiratory Diseases and on Air-Borne Infections carried out in an army camp showed that during the period of low incidence of respiratory infection fewer cases occurred in oiled than in non-oiled areas, while at the height of the incidence of respiratory disease there was no relative difference in the number of cases arising in the two areas (80).

Another illustration of the effect of the application of oiling procedure, in a civilian hospital, is furnished by the experience gathered in the Chicago Lying-in-Hospital (University of Chicago Clinics) (46). Cultures of air were taken during one month prior to oiling; beginning the following month, all articles coming into the laundry, with the exception of personal clothing, were subjected to oil treatment. The next month, another set of cultures were taken on the same premises and under the same conditions. The following two graphs (Figs. II and III) show the results obtained. The first describes the effect in terms of the numbers of bacteria per cubic foot of air, the second does it in terms of numbers of bacteria per settling plate (blood agar) exposed for one hour; the latter method takes into account those bacterial particles which remain suspended for relatively long periods.

The interpretation of these findings depends somewhat upon one's own point of view. Certainly a decrease of the bacterial count by 90 and 84 per cent respectively, in a four-bed ward, is considerable. On the other hand, reductions of the order of 50 percent in a

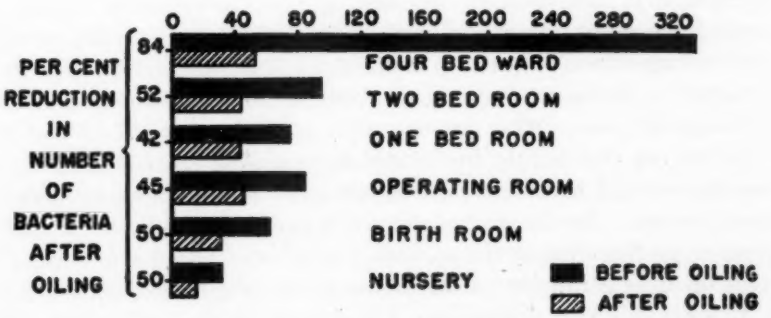
two-bed room are decidedly less impressive, and the results relating to the one-bed room conditions are rather difficult to explain. The situation with respect to the operating room is hardly satisfactory.

FIG. II.



Number of bacteria per cubic foot of air. Reduction in the bacterial content of the air as a result of application of oil to environment. Air-borne bacteria collected by the bubbler sampler. (Robertson)

FIG. III.



Number of bacteria per settling plate exposed for one hour. Reduction in the bacterial content of the air as a result of application of oil to environment. Air-borne bacteria collected on blood agar plates (Robertson).

Thus it may be questioned as to whether the oiling procedure should be regarded as the solution of the problem of transmission of respiratory infection by dust-borne bacteria. Unfortunately, only scanty information is available concerning the performance of disinfectants under comparable conditions. While Crosbie and Wright (44) refer to oiling as an aid against the bacterial contamination of air, and to scrubbing as being incapable of reducing the bacterial content of dust, they report that mopping with a solution of a coal tar disinfectant eliminated a heavy infection of the dust with diphtheria organisms of the "gravis" type. Realizing that bacteria laden dust constitutes a relatively concentrated and persistent focus of infection, Crosbie and Wright visualized the ideal disinfectant for this purpose as one which would have the physical properties of spindle oil, but would kill the microorganisms while holding them down. In their opinion, currently practiced procedures fail to eliminate bacteria in floor dust. It is too early to say whether a formula consisting of a diluted solution of urea, a non-ionic detergent and a cationic disinfectant which was tried out in this country recently (39) will answer the requirements of such a disinfectant.

Comment on Oiling Procedure and Inferences

At any rate, the several findings referred to before certainly appear to reestablish and even to expand the need of surface disinfection. But they require also a revision of some fundamental ideas regarding the types of disinfectants needed under the conditions described, and their capacity of performance. In this connection, is one justified in assuming that the extensive application of an inert mineral oil rather than that of an antibacterial chemical agent was resorted to, both here and abroad, simply because a suitable disinfectant was not available in adequate supply and at a reasonable price? This question must remain unanswered. But it is fair to say that hardly any of the then existing types of bulk disinfectants would have lent itself satisfactorily for the purposes under consideration. In the first place, the suppression of bacterial survival or proliferation in the secondary reservoirs calls for a substantially odorless germicide, or one possessed of an agreeable odor, since a strongly odorous disinfectant (e. g., one of the crude coal tar variety) is bound to become objectionable to the patient or to the hospital staff, who may be exposed to its smell more or less con-

tinuously for a long period of time. Secondly, this type of product should lend itself to the formulation of use solutions (from readily available ingredients other than water) which would satisfy the requirement as to a capacity of effective and prolonged suppression of floor and other environmental dust. Thirdly, such a product should have undergone adequate testing to show that, when used as directed, it is capable of killing resistant strains of streptococci and staphylococci not only in broth but also when dried in mucus, and mixed with dust, lint, etc. Admittedly, there are as yet no suitable testing methods simulating the latter set of conditions; but there is no reason why research could not be directed toward the development of such needed methods.

By determining the capacity of practical performance of such antibacterial compositions from truly relevant experimental premises, rather than by guessing at it from the results of less relevant "short-cut" laboratory tests, chemical surface disinfectants could probably contribute one more effective weapon for the control of air-borne infections.

This idea is not intended, of course, to detract from the position of those formulations whose combined antibacterial and deodorant action renders them peculiarly suitable for the grosser, less articulated purposes of disinfection. It is intended to emphasize the need for recognition of the limitation of required antibacterial performance, depending upon the presence in the formulas of such ingredients as, e. g., hydrocarbon oil, pine oil, terpineol, etc. which tend to affect their activity especially with respect to gram-positive cocci. As indicated before, the phenol coefficient determined with *E. typhosa* as test organism supplies no information on the latter point; the so-called "5 percent phenol" or "20 times the coefficient" safety factor is not much more than an arbitrary short cut, devoid of any accuracy or methodological justification. Incidentally, this contention does not necessarily contradict the findings reported by Varley and Reddish (81) to the effect that dilutions of coal tar disinfectants prepared according to the "20 times the phenol coefficient" rule are capable of disinfecting surfaces artifically contaminated with broth cultures of several pathogens, since these findings are not sufficiently comprehensive in regard to either the many different types of disinfectants commercially available today, or to the conditions under which bacteria

concerned with air-borne infection must be eliminated. It is held that a fresh start should be made in order to secure experimental support for the role which chemical disinfection evidently is called upon to play in connection with this most recent aspect of the control of contagious disease.

The Disinfectant Supply Picture

At this point, a few remarks may be in order with regard to the availability of disinfectants (82).

Broadly speaking, the most important available disinfectants fall in the following major categories: coal tar disinfectants (including the soluble cresylic and emulsifiable tar oil sub-groups), pine oil disinfectants (including the newer terpineol variety), synthetic phenolic disinfectants (derived from alkyl, aryl, alkaryl and halogen phenol derivatives as well as combinations), and quaternary ammonium disinfectants.

Although the coal tar disinfectants have been used most extensively, with the cresylic disinfectants playing the dominant role particularly in hospitals as well as in general medical practice, the recent war and post-war developments have changed this situation to a marked degree.

The yield of coal tar products generally depends upon the output of the tar itself, and the latter in turn depends upon the manner of operation of coking plants which supply the bulk of tar production. Because of the tremendous demands of the steel industry, the coking ovens are being run in short cycles aimed at a maximum output of coke. In addition, owing to the increase in the price of petroleum fuel for heating the ovens, the coking plants have begun to use the tar as fuel, in the interest of operating economy. Both these factors are contributing to a stringency of raw materials, and particularly of cresylic acid, needed for the formulation of disinfectants of the pharmacopoeial and related varieties. Increasing quantities of this type of tar acid are being deflected also to other industries, notably for the manufacture of plastics and related products. As a result, fortification of coal-tar disinfectants with synthethic phenol derivatives is being resorted to, although there is ample evidence to show that the employment of certain synthetic phenolic disinfectants is fully justified upon their own merits, rather than as substitutes for other materials in short supply.

Since several available synthetic phenol derivatives can be produced in a satisfactory state of purity, as characterized particularly by freedom from objectionable odor, disinfectants properly formulated with them should lend themselves especially for use in connection with the practical control of dust-borne infection which has been considered in some detail before.

The literature on disinfectants of phenolic character, both of coal tar and of synthetic origin is very extensive. It would be impossible to discuss even the most important aspects of this chapter within the limits of this presentation. Besides, it is felt that any pertinent investigations published in the recent past, are not likely to affect their present position in the disinfectant picture. This is why it is deemed preferable to select for discussion the newer class of the quaternary ammonium compounds which, in its various aspects, has been receiving considerable attention from different investigators in the recent past.

Quaternary Ammonium Disinfectants

Comprehensive reviews of published literature on the subject of quaternary ammonium compounds have been prepared by Rahn and Van Esseltine (83), and Glassman (84), and should be consulted for the coverage by a specific paper of any aspect of this subject upon which information is desired. This presentation will concern itself with certain limiting considerations which apply to the class of quaternary ammonium disinfectants in the light of certain recent data.

By way of a general remark, it may be said that the literature on disinfectants and antiseptics of all kinds frequently yields examples of a tendency to emphasize the efficacy of a given substance in terms of its performance under certain selected laboratory conditions, but without adequate reference to the limiting effects of other conditions whose application might have been justified by considerations of an existing or intended practical use. While such considerations need not necessarily apply in the case of a strictly theoretical inquiry, e. g., of one attempting to correlate the chemical configuration of a series of structurally related compounds with their anti-bacterial action, they certainly should apply in those cases in which the experimental data are to serve as a basis for the practical use of a given disinfectant or antiseptic agent. An apparent neglect on the

part of different investigators to pay due attention to this factor prompted Klarmann and Wright (85) to embark upon a study of the effect of organic matter upon the germicidal performance of a number of disinfectants, among which were also two products of the quaternary ammonium type. The following Table IV shows that organic matter in the form of 10 percent horse serum, or of 10 percent horse blood, affects the germicidal performance of these quaternary ammonium compounds much more profoundly than that of phenol; thus, whereas phenol appeared to retain 80 or 90 percent of its activity in the presence of this organic material (as compared with the results obtained in its absence), the residual activity with respect to *E. typhosa* of one of the quaternaries was only 3 and 0.6 percent in the presence of 10 percent serum and 10 percent blood, respectively, while the corresponding figures for the other quaternary were 15 and 10 percent. Rather different results were observed with the two products when *S. aureus* was used as test organism, a fact

TABLE IV

PERCENTAGES OF GERMICIDAL ACTIVITY LEFT IN THE PRESENCE OF ORGANIC MATTER (KLARMANN AND WRIGHT)

Disinfectant	*Eberthella typhosa* % Activity in presence of		*Staphylococcus aureus* % Activity in presence of	
	Serum	Blood	Serum	Blood
Phenol	90	90	80	80
Cresol Comp. U. S. P.	80	50	65	50
Cresylic disinfectant (Stated phenol coeff. 5)	90	60	50	45
Fortified cresylic disinfectant (Stated phenol coeff. 5)	80	30	50	25
Orthophenylphenol 15% disinfectant (Saponaceous)	80	20	30	10
Synthetic phenolic disinfectant (Stated phenol coeff. 10)	75	15	25	10
4-Chlorophenylphenol 8% disinfectant	40	15	15	2
Coal tar disinfectant emulsifiable (Stated phenol coeff. 6)	70	70	80	65
Pine oil disinfectant (Stated phenol coeff. 4)	90	100	65	100
Chlorine disinfectant I	1.5	0	1	0
Chlorine disinfectant II	1.5	0.5	1.2	0.25
Quaternary ammonium disinfectant (I)	3	0.6	25	15
Quaternary ammonium disinfectant (II)	15	10	80	45

which illustrates, among other things, the hazard involved in any attempt to treat this class of products as a unit. The other figures illustrate the pertinent conditions found in the cases of other representative types of disinfectants among which is the cresol compound of the U. S. Pharmacopoeia, certain cresylic and synthetic phenolic compositions, as well as products containing respectively tar oil, pine oil and chlorine as active principles.

Evaluating the Germicidal Potency of Quaternary Ammonium Compounds

Failure to obtain consistent results when testing quaternary ammonium compounds by the F. D. A. method, and the apparent inability of other investigators to check each others' as well as their own results (86, 87) suggested to Klarmann and Wright a broader inquiry into the antibacterial behavior of this class; this led them to several conclusions among which are the following:

a) the original F. D. A. testing method is not the proper procedure for evaluating this class of compounds,

b) the quantitative results (including phenol coefficients) obtained by the original F. D. A. method are erroneous from the point of view of the basic principles underlying the operation of this method,

c) a comparison of quaternary ammonium disinfectants with other classes of disinfectants in regard to their respective anti-bacterial performance will yield incorrect information if based solely upon the results of F. D. A. tests,

d) it is possible to determine the germicidal action of quaternary ammonium compounds, as it is understood by the F. D. A. method, with the aid of suitable modifications designed to take care of their peculiarities.

The premises from which these conclusions are drawn will be found in the original papers; suffice it to say, at this point, that the following two factors have been identified as being primarily responsible for the inability of the original F. D. A. method to serve for the evaluation of the quaternary ammonium compounds:

1) the creation of a condition in the "medication" mixture (of the disinfectant dilution plus bacterial inoculum) which prevents the transfer of a truly representative bacterial sample into the subculture,

2) the failure to take account of, and to suppress the characteristic and marked bacteriostatic action of these compounds in the transfer tubes.

The following Table V illustrates the divergence of results obtained in the case of four quaternary ammonium compounds and one long-chain primary alkyl ammonium salt when tested respectively by the original F. D. A. procedure and a "semi-micro" modification (using *E. typhosa* as test-organism), and the practical absence of such a divergence in the case of phenolic disinfectants drawn upon for comparative purposes. (Pine oil and tar oil preparations are affected although not to the same extent as the quaternary ammonium

TABLE V

APPLICATION OF "SEMI-MICRO" TECHNIQUE TO DIFFERENT CLASSES OF DISINFECTANTS (*E. typhosa*, 20° C.) (KLARMANN AND WRIGHT)

Disinfectant	F. D. A. Technique Minimum Concentration Germicidal in 10 min.	Phenol Coefficient	"Semi-Micro" Technique Minimum Concentration Germicidal in 10 min.	Phenol Coefficient
Phenol	1:80		1:70 (1:80)	
Cresol Compound U. S. P.	1:160	2	1:140	2
Cresylic Disinfectant (declared p. c. 5)	1:500	6.2	1:400	5.7
Synthetic Phenolic Disinfectant (declared p. c. 5)	1:600	7.5	1:500	7.1
Synthetic Phenolic Disinfectant (declared p. c. 10)	1:800	10	1:700	8.7
Pine Oil Disinfectant (declared p. c. 4)	1:300	3.7	1:150	2.1
Coal Tar Disinfectant (declared p. c. 7.5)	1:700	8.7	1:400	5.7
Dodecylamine (10%)	1:1500	18.5	1:300	4.2
Quaternary Ammonium Compound A (10%)	1:1500	18.5	1:300	4.2
Quaternary Ammonium Compound B (10%)	1:800	8.8	1:100	1.2
Quaternary Ammonium Compound C (10%)	1:1500	18.5	1:100	1.2
Quaternary Ammonium Compound D (0.1%)	1:15	0.18	1:2	0.03

compounds.) The situation with respect to *S. aureus* is shown in Table VI; because of the extraordinary bacteriostatic action of these substances upon *S. aureus,* substantial quantities of serum are required to combat this effect although its complete suppression is not always possible.

More recently, Klarmann and Wright introduced another refinement into their "semi-micro" method for testing quaternary ammonium disinfectants which depends upon the use of Bacto-oxgall as "suppressor" of bacteriostasis by quaternaries (88). Nutrient broth containing Bacto-oxgall is used to dilute the entire "medication" mixture at the end of the test period in order to stop both the bactericidal and bacteriostatic actions. (In the "semi-micro" method, the volume of the medication mixture measures only one-tenth of the corresponding volume of the original F. D. A. method.) By means of this procedure, one obtains a set of figures as shown in Table VII which contrasts the F. D. A. phenol coefficient figures claimed for the several quaternary ammonium compounds with those obtained by the refined "semi-micro" method.

In attempting to account for the reason why representative numbers of bacteria are not transferred from the medication mixture

TABLE VI

APPLICATION OF "SEMI-MICRO" TECHNIQUE TO DIFFERENT CLASSES OF DISINFECTANTS (*S. aureus,* 20° C.) (KLARMANN AND WRIGHT)

Disinfectant	Minimum Concentration Germicidal in 10 min	Minimum Concentration Germicidal in 10 minutes			
		F. D. A. Broth	Vol. of broth		Serum Broth Vol. of 50% serum
Phenol	1:60	1:50	20 cc.	1:50	40 cc.
Cresol Comp. U.S.P.	1:90	1:80	20 cc.	1:70	40 cc.
Synthetic Phenolic Dis. II	1:250	1:175	20 cc.	1:150	40 cc.
Quat. Ammonium Comp. A 10% ...	1:1500	1:500[1]	200 cc.	1:300[2]	40 cc. of whole serum
Quat. Ammonium D. 0.1%	1:30	1:5[1]	200 cc.	1:2[2]	40 cc. permits of growth whole serum

1. Complete inhibition
2. Partial inhibition

into the subculture, Klarmann and Wright proceeded from the hypothesis that in the presence of quaternary ammonium compounds, the viable bacterial cells are not uniformly distributed in the liquid, but that they tend to be massed against the walls of the containing tubes. They assumed further that this might be due to the neutralization of the negative charges of the suspended cells resulting in the formation of a precipitate with a tendency to adhere to a solid surface. They carried out a number of exploratory experiments in support of this hypothesis, using *Ps. aeruginosa, E. typhosa* and *S. aureus* as test subjects. More recently, Kivela, Mallmann and Churchill (89) observed clumping in the presence of a quaternary ammonium compound with *Ps. aeruginosa* and *Es. coli,* but not with *E. typhosa* or *S. aureus* (possibly because of experimental conditions differing from those employed by Klarmann and Wright). Using bacterial spores, they showed also that the bacteriostatic effect of quaternary ammonium compounds could be reversed by diluting and agitating the spore suspension with water or physiological saline. They regard the high osmotic pressure produced upon the bacterial cell wall by the adsorbed quaternary ammonium disinfectant as one of the principles of the latter's anti-bacterial effect, and incidentally as a factor in Hotchkiss' observation of the discharge of the cell fluids of vegetative bacteria into the suspending medium (90). The reversal in the case of spores is accounted for by their cell wall which is much thicker than that of the vegetative bacterial cells, and consequently more resistant to osmotic effects.

TABLE VII

"Semi-Micro" Method Employing Bacto-Oxgall (B. O. G.) (Klarmann and Wright)

Disinfectant	*E. typhosa* Phenol Coeff. F. D. A. Method	*E. typhosa* Phenol Coeff. Semi-Micro B. O. G.	*S. aureus* Phenol Coeff. F. D. A. Method	*S. aureus* Phenol Coeff. Semi-Micro B. O. G.
Quaternary Ammonium A (10%)	25	23	27.9	2.3
Quaternary Ammonium B (10%)	19	Not Germicidal	23	1.5
Quaternary Ammonium C (10%)	20	1.4	40	3.2
Quaternary Ammonium D (10%)	18	Net Germicidal	50	0.9
Dodecylamine (10%) ...	15	1.3	15	0.6
Phenol	1	1	1	1

While a reversal of the bactericidal action of a quaternary ammonium compound (i. e., of a cationic agent) by an anionic agent (e. g., sodium lauryl sulfate) has been claimed by Valko and Dubois (91), it follows from a more recent investigation by Klein and Kardon (92) that this is not a case of true reversal, but one of inactivation of the cationic by the anionic agent, and of the consequences of such inactivation.

A rather extensive investigation into the mechanism of the anti-bacterial action of quaternary ammonium compounds has been carried out by McCulloch and coworkers (93, 94). Proceeding from the observation that the plate counts of a culture exposed to the action of a cationic disinfectant decrease rapidly during the first few minutes, but less so as the experiment progresses, they assume that the initial decrease is due to clumping while the subsequent slow decrease, after several hours, depends upon the theoretical ability of a clump to give rise to a bacterial colony if it contains at least one viable cell. This effect is illustrated by the following graph (Fig. IV),

FIG. IV

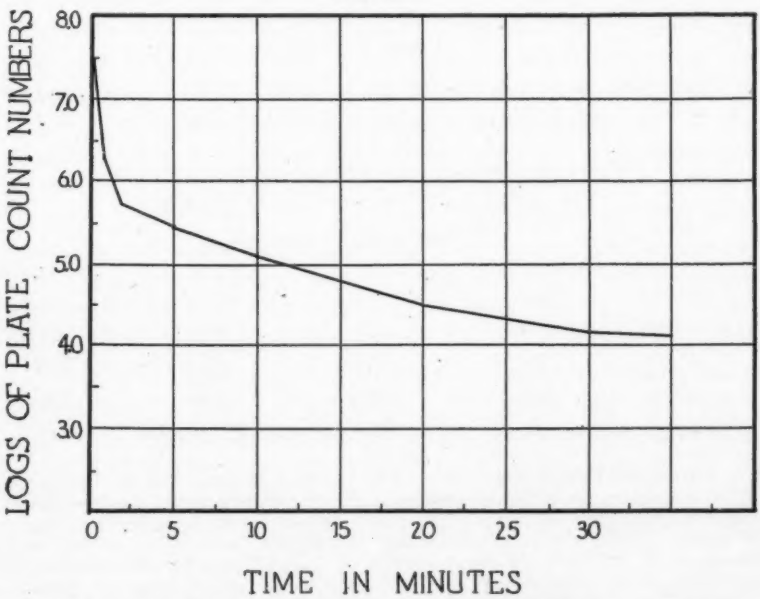


Plate counts of *S. aureus* under the action of a quaternary ammonium compound (McCulloch).

in which the logarithms of the plate count numbers are plotted against the time in minutes; *S. aureus* is the test organism.

This characteristic behavior, as well as the strongly impairing action of organic matter (such as milk, blood, etc.) upon the anti-bacterial performance of quaternary ammonium compounds, are held to furnish adequate reasons for the limitation of uses to which these compounds are suited. McCulloch, too, considers the F. D. A. phenol coefficient values of quaternary ammonium compounds as misleading, and urges their omission in labeling and advertising.*

In preparing aqueous solutions, it is necessary to keep in mind also the extent to which tap water of different localities may affect the anti-bacterial action of quaternaries. A limited illustration of this problem is furnished by the following Table VIII. A more complete study of the pertinent effects of calcium and magnesium hardness has been carried out by Ridenour and Armbruster (95). With respect to the sanitizing (as opposed to the disinfectant) uses of quaternary ammonium compounds, these authors, too, caution against their promiscuous application and recommend adjustment of

TABLE VIII

EFFECT OF TAP WATER ON REQUIREMENTS FOR QUATERNARY AMMONIUM COMPOUNDS (McCULLOCH).

	Parts per million required to produce a 100% kill of Esch. coli in one minute when the compound is dissolved in			
Compound Designated	*Pure water*	*Cincinnati tap*	*Norwood tap*	*Parts per million recommended for use by producer*
A	20	100	250	234
B	10	70	250	190
C	5	500	3,200 plus	78

* Mallman, Leavitt and Joslyn (168) reported recently that the susceptibility of *S. aureus* to the action of quaternary ammonium compounds depends, to a major degree, upon the composition of the nutrient broth in which this organism is grown. Thus the same quaternary disinfectant kills an *S. aureus* culture grown in F. D. A. broth in a dilution of 1:20,000 (in 10 minutes), and one grown in Difco "disinfectant testing" broth in the much stronger dilution of 1:4000. No such effect was observed with phenol and with two disinfectants of phenolic character.

the concentration factor to the specific conditions of use in order to insure proper sanitization.

Unquestionably, the quaternary ammonium compounds have their proper and important place in the disinfectant picture, since they are capable of performing a multitude of useful and special tasks, if applied intelligently. By the same token, failure to keep some of their typical properties in mind may involve certain risks. A misconception regarding their anti-bacterial capacity, as drawn, e. g., from the results of an ill-suited laboratory testing technique might conceivably create one such risk. Adequate directions for use, derived from representative tests reflecting the conditions to be encountered in the practices of disinfection and sanitization would minimize this risk.

Bactericidal vs. Bacteriostatic Action of Antiseptics

In the field of antiseptics, like in that of disinfectants, there is evidence of a tendency to break away from formalized testing procedures, and to determine the merits of the individual antiseptics on the basis of their performance in actual practice, or in tests which adequately approach the conditions of practical use. In testing antiseptics as to their fitness for specific purposes many factors may have to be considered which are not revealed by a routine laboratory procedure, such as the F. D. A. method. In all fairness to the latter it should be said, however, that its authors were completely aware of the inability of any laboratory test to duplicate the conditions found in practice, and that the several procedures outlined in Circular No. 198 were intended primarily to uncover the obviously inactive and useless preparations. Among other things, this document refers to the danger of obtaining false values for products with high inhibitory action, such as the mercurials, and it suggests Shippen's (96) simple expedient of distinguishing between bactericidal and bacteriostatic actions. Incidentally, Heinemann (97) pointed out more recently that the dilution principle of Shippen's technique is not always sufficient, in that even by this modification falsely high killing results are obtained. A corresponding observation (with respect to quaternary ammonium compounds) was reported by Klarmann and Wright (85).

Since the publication of the F. D. A. method in 1931, a considerable number of experimental investigations have been carried

out which emphasized the need of more critical tests for antiseptics intended particularly for clinical or surgical use. In connection with organic mercurials (which as a class seem to enjoy professional acceptance in the form of both aqueous solutions and alcoholic tinctures) a critical point appears to have been reached with the recent publication of a paper by Morton, North and Engle (98) who showed that virulent hemolytic streptococci remain infectious after exposure for ten minutes to any of the three commercially available mercurials: merbromin N. F., sodium ethylmercurithio-salicylate and 4-nitro-3-hydroxymercuri-orthocresol anhydride. In performing their tests, Morton and coworkers used a streptococcus culture of such virulence that one cubic centimeter of a dilution of 1:10,000,000 killed white mice when injected intraperitoneally.

Of course, the question as to the distinction between the bactericidal and bacteriostatic action of mercurials has been raised before. As early as in 1889, Geppert disproved Koch's claim that mercuric chloride was capable of killing anthrax spores (99); in fact, using ammonium sulfide to suppress bacteriostasis, he showed that a 0.1% solution of mercuric chloride did not kill anthrax spores even in 24 hours.

One of the highlights in a number of subsequent pertinent developments is the introduction in 1940 of the thioglycolate medium by Brewer (100) which neutralizes the bacteriostasis produced by mercurials (101). This type of medium is now being used extensively; one such formulation has been made official by the National Institute of Health (102) in connection with tests for sterility of biological materials.

A direct inquiry into the capacity of antiseptics to prevent infection *via* cutaneous lesions was carried out almost simultaneously, but independently, by Nungester and Kempf (103), and by Sarber (104). In this connection, it has been emphasized that a statistical evaluation of clinical experience with antiseptics intended e. g., for preoperative use is not conclusive, for the following reasons: firstly, potentially pathogenic microorganisms are not always present on the skin, and secondly the resistance of tissue to infection is high, especially in the absence of any extensive traumatization. Thus a substantial number of successful operations accompanied by a low incidence of wound infection would lead to the erroneous conclusion that the credit is due to the antiseptic used, when, in all probability,

the same or a similar situation would have resulted if no preoperative antiseptic had been employed.

This is why Nungester's and Kempf's, as well as Sarber's methods provide for the intentional implantation of a portion of infected and subsequently treated skin within the peritoneal cavity of an animal. In the former's method, the tail is first contaminated with a suspension of streptococci or pneumococci, then treated with the antiseptic solution under test whereupon a portion is cut off and embedded in the peritoneum of an anesthetized mouse. Sarber's method is essentially similar except that a piece of the contaminated and treated skin is excised and introduced into the animal's body.

The following Table IX illustrates the results obtained by Sarber. Of the several antiseptics tested, tincture of iodine appears to have freed the skin of hemolytic streptococci so as to allow survival of 24 out of 25 mice in the case of functioning tissue, and of 21 out of 25 mice in that of non-functioning tissue. (The difference between these two types of tissue depends upon the removal of the former from a live animal while the latter was excised from a mouse which

TABLE IX

PERFORMANCE OF SEVERAL ANTISEPTICS FOLLOWING INTRAPERITONEAL IMPLANTATION OF INFECTED AND TREATED SKIN PORTIONS (SARBER)

(A) The germicidal action of various substances in the presence of functioning tissue.

Germicide	Number Tested	No. of Animals Living in x Days 1	2	3	4	5	6	7
Iodine (tinct.)	25	24	24	24	24	24	24	24
Sodium ethyl-mercury thiosalicylate (1:1000 tinct.)	25	13	13	12	12	12	12	12
Hexylresorcinol (1:000 soln.)	25	20	18	14	10	9	9	9
Cetylpyridinium chloride (1:200 tinct.)	25	18	16	15	14	14	14	14
Control	25	1	0	0	0	0	0	0

(B) The germicidal action of various substances in the presence of nonfunctioning tissue.

Germicide	Number Tested	No. of Animals Living in x Days 1	2	3	4	5	6	7
Iodine (tinct.)	24	22	22	21	21	21	21	21
Sodium ethyl-mercury thiosalicylate (1:1000 tinct.)	24	17	13	11	11	9	9	9
Hexylresorcinol (1:1000 soln.)	24	11	10	10	9	8	8	8
Cetylpyridinium chloride (1:200 tinct.)	24	14	12	12	11	11	11	11
Control	24	0	0	0	0	0	0	0

had been dead for thirty minutes prior to contamination of the skin and treatment with the antiseptic; the excised piece was implanted in the peritoneum of another mouse). The organic mercurials, hexylresorcinol and a quaternary ammonium solution saved the animals in a smaller number of cases; with respect to the last named antiseptic it should be noted that its 0.5% concentration is substantially higher than that of some other preparations of the same class and recommended for preoperative use (frequently in 0.1% dilutions).

Nungester's and Kempf's technique was used by Gershenfeld and Miller (105) who found that two and five percent iodine solutions, and two and seven percent iodine tinctures protected mice against infection with a hemolytic streptococcus which in the control animals caused a mortality of seventy percent.

Pierce and Tilden (106) employed the same technique using pneumococci for their infection experiments. They found the solutions of respectively two percent of phenol, three percent of saponated cresol, two percent of iodine and one percent of a quaternary ammonium compound (dimethyl-benzyl-lauryl-ammonium chloride) to protect mice against pneumonia. (The latter solution appears exceptionally concentrated for this type of compound.) Mercurials were rather ineffective whereas seventy percent ethyl alcohol and concentrated isopropyl alcohol were responsible for less than twenty percent mortality. Preliminary tests were carried out with *M. tuberculosis* as infective organism, and guinea pigs as test animals. A one percent solution of phenol, a two percent of saponated cresol and a one percent of the quaternary ammonium compound were effective in preventing tuberculosis (following intraperitoneal injection of the medicated culture).

Incidentally, Nungester, Hood and Warren checked the results of their "infection-prevention" method by means of an *in vitro* procedure employing thioglycolate media (107). While with the nonmercurial antiseptics essentially the same results were obtained, whether or not thioglycolate was used in the medium, the percentage of positive growths in the case of the mercurials was very much greater when subtransfers were made to the thioglycolate containing media. This was considered another indication of the inadequacy of these substances as skin germicides.

At this point it might be interposed that neither Sarber's, nor Nungester's and Kempf's methods give any consideration to inhibi-

tory antiseptics which because of their prolonged contact with the skin might be clinically acceptable. The answer to this argument is that in the case of wound infections one is not concerned, of course, with the microorganisms populating the unbroken skin, but with those invading the deeper tissues where the bacteriostatic effect may be impaired by dilution as well as by the tissue fluids themselves (blood, lymph); with reference to the mercurials, one must remember the existence of those tissue constitutents which contain sulfur in organic form, and which might, therefore, suppress the bacteriostatic action of the mercurials in a manner analogous to that of thioglycolate employed in the laboratory test.

In 1943, the Council on Pharmacy and Chemistry of the American Medical Association suggested the use of the Nungester-Kempf "infection-prevention method" for the evaluation of any new skin disinfectants (108).

For the sake of completeness, brief reference should be made here to the use of chick embryos in connection with testing wound antiseptics. Green and Birkeland (109) employed eggs containing eleven day old embryos which they infected with *S. aureus* at the chorio-allantoic membrane, on the twelfth day; for six days thereafter they dropped the antiseptic under test upon the membrane, and after the last treatment they swabbed the membrane with a moist cotton swab which was then used to streak an agar plate. The number of organisms recovered in this fashion is considered an indication of the degree of infection. While phenol, azochloramide and two mercurials were not particularly effective, penicillin and three quaternary ammonium compounds gave evidence of efficacy. Since evidently no attempt was made to take care of the bacteriostatic action of the portions picked up and transferred by the swab it is hardly feasible to accept these findings without some reservation.

Role of Gram-Negative Organisms

In addition to the problem of bactericidal vs. bacteriostatic action in connection with wound antisepsis, another problem is that of specificity vs. non-specificity of the antiseptic agent with respect to the invading microorganism. For a long time *S. aureus* held a practically undisputed position as the test-organism *par excellence* for use in the *in vitro* evaluation of formulations intended for antiseptic use; its role in the F. D. A. testing method undoubtedly contributed

to this situation. As a result, particular consideration was given in the laboratory to the aspect of infection with gram-positive microorganisms of which *S. aureus* is a good representative (from several points of view, e. g., ease of laboratory cultivation, susceptibility to standardization of resistance, simplicity of identification etc.). At the same time, less attention was being paid to gram-negative organisms. However, this situation appears to call for a change, in view of the paper by Meleney, Johnson, Pulaski and Colonna (110), for two reasons: firstly, because it reemphasizes the importance of gram-negative bacteria, particularly in mixed infections, and secondly because it points to the indirect role which the gram-negative bacteria can play in the inactivation of locally applied antibiotic agents, such as penicillin. To produce the latter effect, the gram-negative organisms need not even be pathogenic so long as they possess the capacity of multiplying in the presence of wound exudate, and of using the material present in the wound for the elaboration of metabolic products which are antagonistic to the action of penicillin. One such agent, viz., penicillinase, has been proved by Woodruff and Foster (111) to be of enzymatic character; hence its name. Among the different pathogenic bacteria which produce this enzyme is the large group of aerobic non-spore forming gram-negative organisms, and especially *E. coli, B. proteus* and *Ps. aeruginosa.* Rapid inactivation of penicillin is produced also by the group of achromobacters, by gram-negative micrococci (heretofore regarded as unimportant) and by the majority of aerobic gram-positive spore formers. In any case, if the bacteriostatic level of penicillin cannot be maintained in a wound harboring a mixed infection, due to the production therein of penicillinase, it is possible that the multiplication of such organisms as hemolytic streptococci and staphylococci will not be inhibited, and that, moreover, these originally penicillin susceptible organisms will have been rendered penicillin fast.

Of some two-hundred chemical and antibiotic agents tested for their effect on aerobic gram-negative bacteria, the great majority selected on the basis of *in vitro* results, were not satisfactory for a variety of reasons when put to the clinical test. Eventually, the choice narrowed down to the antibiotics, streptothricin and streptomycin, and to the chemicals, 9-aminoacridine hydrochloride, 5-nitro-2-furaldehyde semicarbazone and p-chlorophenol. The use of the latter chemical was suggested by Klarmann (112) who also furnished some

of the original ointment formulations employing it; this recommendation was based upon the observed non-specific performance of p-chlorophenol with respect to a representative number of pathogens, coupled with considerations of practical availability and sufficient solubility.

Meleney and coworkers found that a 0.1% solution of p-chlorophenol was inhibitory for almost all of one-hundred and forty strains of freshly isolated gram-negative bacteria, as illustrated by the following Table X. It was employed in most cases in a concentration of 0.25%, usually in an ointment composed of carbowax and propylene glycol. When penicillin was to be used with it, it was incorporated to the extent of 1000 units per gram.

The p-chlorophenol ointment gave no evidence of toxicity, nor of any appreciable retardation of wound healing, as measured by Howes' technique (113) upon experimental wounds. It was used successfully by itself in a number of surgical cases complicated by infections with gram-negative organisms (e. g., *Ps. aeruginosa*), and in combination with penicillin in treating mixed infections of wounds.

TABLE X

BACTERIOSTATIC ACTION OF P-CHLOROPHENOL ON GRAM-NEGATIVE BACTERIA (MELENY ET AL.)

Organism	Strains	Concentration Necessary for Complete Bacteriostasis in Beef Infusion Broth 1:1,000	1:2,000	1:5,000
E. coli	44	5	28	11
Coli intermediates	10	0	9	1
Aerobacter	6	0	5	1
B. pyocyaneus	36	5	19	12
Proteus	28	3	23	2
Achromobacter	5	1	0	4
B. alkaligenes	3	0	0	3
S. alcalescens	2	0	1	1
E. typhosa	2	0	1	1
K. friedländeri	1	0	0	1
Micrococcus	3	0	0	3

(To be concluded next month)

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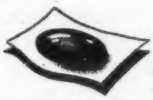

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